Acephatemet artificial antigen method of preparation

The invention relates to the method of utilizing the protein phosphorylation, with the very active forebody result O in acephatemet (O, S- dimethyl thiophosphoryl ammonia) the production process, the method of S- dimethyl thiophosphoryl chloride and the direct crosslinked of carrier molecule (protein) preparation acephatemet artificial antigen belongs to the immunology technical field.

For making nos immunogenic micro molecule material (often being called the hapten, Hapten) can bring out production antibody in animal body in the immunology field, often will with this kind of hapten with certain macromolecule carrier if crosslinkeds such as protein or polypeptide combine to form to the artificial antigen, again the injection advance the mouse and bring out production antibody, after the purification antibody, can be used for the immunoassay, it is one of field the most active in clinical medicine measuring at present and the food retention analysis.

As everyone knowsEverybody knows, the acephatemet is both at home and abroad a widely used hypertoxicity, high-efficient organophosphorus pesticide, once is the biggest pesticide of China's production tonnage, has strong tagging and the stomach poison function to the insect, also has very big harmfulness, some country's prohibitions of use to people and animals. There is the original code restriction in China to the use of acephatemet pesticide, forbids using on short-term crop, if forbid using on the vegetable crop.

Nevertheless remain the toxic phenomenon that too high food led to the fact because of edible acephatemet and still happen occasionally, so its safety monitoring of necessary enhancement is worked.

At present to the detection method of acephatemet gas chromatography, HPLC, single sweep oscilloscopic polarography, thin-layer chromatography method, spectrophotometric method, direct potentiometry etc. as follows mainly, complicated dear instrument need be used to these methods, and loaded down with trivial details pretreatment all will be passed through to every kind of method in addition, hardly reaches quick, simple and convenient witnessed inspections requirement. If can adopt like medically detect similar gold-marking immunity such as early pregnancy, hepatitis B and AIDS or enzyme to ally oneself with immune method, then can reach fast, sensitive and can the remaining needs of witnessed inspections acephatemet.

In order to set up its immunoassay method, at first must obtain the antibody of anti acephatemet. The acephatemet molecular structure is simple, and molecular weight (141) are little, belong to the hapten, directly the immunity produce antibody. Must at first prepare out its complete antigen with its and the coupling of carrier protein matter, just can bring out the animal and produce antibody. We are once many

Inferior trial utilizes the intramolecular amino group of acephatemet to come synthetic acephatemet artificial antigen with classical carbodiimide method (EDC) or glutaraldehyde method, and is nevertheless all unsuccessful. It mainly is because the intramolecular amino of acephatemet is phosphinylidyne ammonia to analyse the reason, is not the primary amine group, unresponsive activated cause. The artificial antigens that the acephatemet has been synthesized to the EDC method for report such as internal Liu Feng power, and scan the mole ratio of extrapolating in the artificial antigen hapten and carrier protein with the UV spectrum and be more than 3000, go through according to our experiment, think that this impossible.

The artificial antigen crosslinked method of a phosphorus compound has been introduced by the State Patent Office in disclosed application number was 94105042.4 patents in June, 95, this patent relates to utilizes bridge construction L- lysine and O- ethyl dichloro phosphate ester condensing agents such as (EDCP) with the method of organo phosphorous compounds hapten with carrier protein matter crosslinked preparation artificial antigen. We with suppressing the experimental specific antibody that almost can not detect anti acephatemet of ELISA, explain that this kind of method is not suitable for the synthesis of acephatemet artificial antigen with the artificial antigen immunity mouse of this kind of method preparation, infer that the reason probably is that the lysine bridge of overlength has weakened the affinity of antibody to the acephatemet because the acephatemet molecule is too simple. And acephatemet one of them intramolecular group is the methylthio, and consequently, the acephatemet molecule is not included to mention within the phosphorus compound scope in this discloses the patent.

The objective of the invention is to seek an appropriate reagent, with the synthetic artificial antigen that goes out the acephatemet of simple, practical method, for the specific antibody of selecting it later on with set up the immunoassay method and provide the basis.

The forebody result O of synthetic acephatemet is selected for use in the invention, and the S- dimethyl vulcanizes amino group on phosphoryl chloride phosphorus oxychloride and the protein in alkaline water solution, under the magetic stirring, direct phosphorylation and coupling, after dialysis and freeze drying the acephatemet artificial antigen, the micro molecule that links on the result has the structure of hapten, a bit configuration that does not destroy and change the hapten. Above-mentioned protein can be bovine serum albumin (BSA), ovalbumin (OVA), the blue albumen (KLH) of blood or people's blood albumin (HSA).

The invention can realize like this:

Join in marriage into 1 to 15% concentration with protein with two water that evaporate, add the triethylamine in the protein solution of the 1-15% concentration of joining in marriage, : the 600-

1000 that the mol ratio that makes protein and triethylamine in 1-15%'s the protein solution be 1 places protein and the mixed solution of triethylamine in the ice bath magetic stirring after 30 minutes, and dropwise the joining in advance

The 0.2-0.5 that the O who doubly dilutes with chloroform or toluene 3-5 earlier, S- dimethyl thiophosphoryl chloride weak solution, O, the addition of S- dimethyl thiophosphoryl chloride weak solution be above-mentioned protein and the mixed solution bulk volume of triethylamine doubly continues ice bath magetic stirring reaction 30 minutes, then will react in liquid changes the separatory funnel over to, stew treat to take out after the layering in the middle of the water layer put into dialysis bag, in 4 DEG Cs of dialysis in distilled water, traded water once in per 4 hours, last freeze drying obtains the acephatemet artificial antigen of synthesis promptly.

The invention has easy and simple to handlely, one step of consummatory response; Do not use the bridge construction, can not produce the antibody of levying the bridge, consequently, make things convenient for the advantages such as screening of specific antibody.

After adding the 1.0ml triethylamine in 10% bovine serum albumin of instance: 8ml (BSA), the magetic stirring is 30 minutes under the ice bath, then dropwise adds the O of 4 times of dilutions of chloroform 2.7ml for, and S- dimethyl thiophosphoryl chloride continues 30 minutes backs of reaction, and reaction liquid changes the water layer over to dialysis bag (by molecular weight 10000) after the layering of stewing in changing a little separatory funnel over to, with distilled water dialysis 3 days, trades water once in per 4 hours among them, the artificial antigen (abbreviation BSAM) that must synthesize the dislysate freeze drying promptly at last. Can be with the synthetic coating antigen of method (being called for short OVAM) with ovalbumin (OVA) replacement BSA.

The appraisal of artificial antigen:

With the concentration of the anti colorimetry of molybdenum antimony survey phosphorus, the hapten in the calculation artificial

antigen and the molecule coupling of carrier protein (BSA) are than being 26.

Artificial immunity antigen BSAM compares many 1027cm in the artificial antigen BSAM infrared spectrogram with former carrier protein BSA infrared spectrogram^{-1}And 789cm^{-1}Two absorption peaks, these two peaks are γ P=O (vs) and γ P-N (s) characteristic absorption peak respectively, further explain the artificial antigen and synthesize successfully.

The immune animal identification has produced the antibody of anti acephatemet: the solution of joining in marriage into 1.0mg/ml with the synthetic BSAM of the aforesaid with 0.8% physiological saline is tested in the immunity of (1) mouse, and immunity is for the first time joined in marriage good BSAM equivalent with 0.5ml complete Freund's adjuvant and the aforesaid and is mixed, after the abundant emulsification, every mouse (Balb/c mouse, 6 week age) injection 0.2ml, 100 μ g protein, 4 mouse of immunity altogether in other words. The every is 2 weeks, uses incomplete Freund ditto method booster immunization 3 times again instead. Mouse afterbody blood, preparation antiserum were got in back 15 days in the 4th immunity. (2),

The coating antigen of 100 μ pH9.6's for the 1 carbonate buffer solution dilution is added in ELISA competition rejection testing every hole in the enzyme designation strip in every 12 hole, includes 2 μ g OVAM, and 37 DEG Cs of constant temperature displace 4 DEG Cs of refrigerators and spend the night after hatching 1h. Take out and give a baby a bath on the third day after its birth time (PBST:PBS 20nmol/L, 0.05%Tween-20, pH7.4) with PBST, spin-dry. Therefrom getting three and putting on the ELIAS plate, add 50 μ l, two anti weak solutions (PBS prepares, includes 0.1% gelatin) in every preceding 1-2 hole (totally 6 holes), the 50 μ l of standard acephatemet liquid that prepare with two anti weak solutions are added in the 3-4 hole, include 5 μ g trade sample acephatemets, and the 5-6 hole includes 10 μ g trade sample acephatemets with the 3-4 hole, the downthehole 20 μ g trade sample acephatemets that contain of 7-8, and the downthehole 100 μ g trade sample acephatemets that

contain of 9-10, 50 μ l, two anti weak solutions are also added in the 11-12 hole, then every 10 preceding holes all add 1: 200 above-mentioned antiserum, the 50 μ l that prepare with two anti weak solutions, and 50 μ l, two anti weak solutions are still added in the 11-12 hole, have added the afrerdischarge and have hatched 1h at 37 DEG Cs of constant temperature water tanks, take out, give a baby a bath on the third day after its birth time with PBST, then every hole add 100 μ l with 1: 5000 goat-anti mouse ELIAS secondary antibody of two anti weak solutions preparation, and 1h is hatched to 37 DEG Cs of constant temperature water tanks, takes out to give a baby a bath on the third day after its birth inferiorly with PBST, adds the o-phenylenediamine and the perhydrol substrate liquid of pH5.0 phosphoric acid - the citric acid preparation for the 1 of 100 μ, after 37 DEG Cs of constant temperature water tanks seal up and place 15min, 50 μ l of every hole joining, 10% sulphuric acid, then read the OD value with the 492nm wavelength on the ELIASA, get the average numerical result in 6 holes as follows:

The competition suppresses the ELISA experimental result

The hole count	1-2	3-4	5-6	7-8	9-10	11-12
The OD value	1.41	1.05	0.88	0.69	0.51	0.08

On show data explanations: the colour of adding the acephatemet hole all more of light color than the hole that does not add the acephatemet with, compares to shine deeply, increases along with what standard acephatemet was added in addition, and the enzyme becomes more and more shallow with the colour of substrate reaction, explain to have the antibody that can react with the acephatemet in the antiserum, thereby prove that the mouse has produced the specific antibody of anti acephatemet, proves further that artifical antigen synthesizes successfully.

Claim

1. acephatemet artificial antigen synthetic method characterized in using O, and amino gene is in alkaline water solution on object and the protein before as the synthesis of this antigen for S-

dimethyl thiophosphoryl chloride, and under the magetic stirring, the triethylamine is added to direct phosphorylation and 1-15% protein solution during the preparation, is joined in marriage into with distilled water for the protein to the synthetic acephatemet artificial antigen of coupling in protein solution,: the 600-1000 that the mol ratio that makes protein and triethylamine in the 1-15% protein solution be 1, and protein solution 30 minutes backs of magetic stirring in the ice bath are dropwise added and are diluted 3-5 0 doubly, and the 0.2-0.5 that S- dimethyl thiophosphoryl chloride weak solution, its addition be protein and the bulk volume of the mixed solution of triethylamine doubly continues ice bath magetic stirring reaction 30 minutes, fetches water after the layering of stewing layer to dialyse 3 days, the acephatemet artificial antigen that must synthesize the dislysate freeze drying promptly again.

2. the acephatemet artificial antigen synthetic method of saying according to claim 1 characterized in that the carrier protein can be bovine serum albumin (BSA), ovalbumin (OVA), the blue albumen (KLH) of blood or people's blood albumin (HSA);

3. the acephatemet artificial antigen synthetic method of saying according to claim 1 characterized in hapten object 0 before synthetic, and the retarder thinner can be made with chloroform or toluene to S- dimethyl thiophosphoryl chloride.